Regulation of Respiration in the Leaves and Roots of Two *Lolium perenne* Populations with Contrasting Mature Leaf Respiration Rates and Crop Yields[1]

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ABSTRACT

Measurements of O_2 uptake were made on leaves and roots of two populations of *Lolium perenne* L. cv S23 (GL66 and GL72), previously shown to have contrasting rates of CO_2 evolution and yields of dry matter. O_2 uptake was faster in the mature leaves of GL66 than those of GL72, but no difference was observed in the respiratory rates of meristematic leaf bases or mature roots. The growth rate of GL72 was faster than that of GL66. Cyanide resistance was substantial in mature leaves but the alternative path did not contribute to O_2 uptake in the dark. In both populations, adding malate and glycine stimulated O_2 uptake, but exogenous sucrose only stimulated when uncoupler was also present. The difference between the respiratory rates of the two populations was maintained under all investigated conditions. We conclude that the rate of mature leaf respiration in the dark in *L. perenne* is limited by adenylate control of glycolysis. The difference between the fast (GL66) and slow (GL72) respiring populations reflected a greater respiratory capacity and higher turnover of ATP in GL66. Alternative path capacity was also high in the roots of both and contributed substantially to O_2 uptake, as indicated by inhibition by salicylhydroxamic acid in the absence of KCN. The alternative path capacity of meristematic leaf bases was considerably less than that in mature leaves.

Transverse and cross-sections were made of mature leaves of both populations to study anatomical features which might explain the differences in ATP turnover, suggested by the biochemical experiments. Leaves of GL72 were thicker but did not show a different anatomy when compared with GL66. The increased thickness was not due to more or larger cells but entirely to a larger intercellular volume.

In many plant species, a significant portion (40-60%) of the carbon fixed daily via photosynthesis is used in dark respiration (9). Consequently, manipulation of the rate of dark respiration is potentially a very productive approach to the improvement of crop yields, particularly in forage grasses where any increase in above-ground biomass is reflected directly in yield. Robson (12, 13) and Wilson *et al.* (15-17) have recently reported significant (up to 13%) increases in the annual yield of *Lolium perenne* L. (perennial ryegrass) plants selected for slower (compared to parental plants) rates of respiration. This increase in biomass was positively correlated only with the rate of mature leaf respiration measured as CO_2 loss in the dark, and not with the rate of photosynthesis or photorespiration (15), and was not associated with any obvious agronomically undesirable characteristics (17).

To date, nothing is known concerning the biochemical nature or regulation of respiration in these plants, although such knowledge is obviously important for future exploitation of respiratory parameters and the detection of possible deleterious side effects of selection for slower respiring lines. In the present paper, we report initial investigations of leaf and root respiration in two *L. perenne* populations previously shown to differ in their rates of dark CO_2 efflux from mature leaves. Particular attention was paid to the relative involvement of the non-energy conserving, alternative pathway of electron transport (10), since this might be expected to affect the respiratory efficiency. Some anatomical investigations of the leaves were carried out, in an attempt to find clues to the nature of the greater turnover of ATP which was indicated by the results of biochemical studies on the respiration of the *Lolium* lines.

MATERIALS AND METHODS

Seed Production and Growth of Plants. Two populations derived from *Lolium perenne* cv S23 were examined. One, GL66, was a polycross progeny obtained by open pollination of nine genotypes selected for fast mature leaf respiration. The other, GL72, was a polycross progeny similarly obtained from 18 genotypes selected for slow respiration (15, 16). GL72 was derived from the same slow respiration families as another population, GL83, whose growth in the field is described by Wilson and Jones (17). Seeds were germinated in vermiculite and the seedlings transferred into nutrient solution (14) about 10 d after sowing. Growth occurred in a controlled environment cabinet (20°C, 16 h d, 250 μmol·m^{-2}·s^{-1}, photosynthetically active radiation). The age of the plants used varied from 15 to 90 d.

Measurements of O_2 Consumption. *Leaves.* Small segments were cut with a razor blade from either the middle portion of fully expanded leaves (mature tissue: 2-mm segments were cut) or from the unexposed bases of young leaves (meristematic tissue: 5-mm segments were cut). For each separate measurement, at least four different leaves were used and cutting was done with the leaves submerged in 20 mM Hepes buffer (pH 6.5), containing 0.2 mM CaCl$_2$. The slices were incubated in the buffer for 30 min

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at room temperature (20–25°C) in the dark, to avoid possible interference from postillumination transients (2). O_2 uptake was measured at 20°C in 5 ml of the above buffer using a Clarke type of O_2 electrode (YSI, Yellow Springs, OH). When effectors were used, they were added directly to the reaction vessel, and O_2 uptake was subsequently followed for 10 to 15 min. SHAM[4] was added from a 1 M solution in methoxy-ethanol; CCCP from a 2 mM solution in ethanol.

Roots. For each measurement, all the intact roots of three different plants were cut and thoroughly rinsed in nutrient solution (see above). O_2 uptake was measured in 100 ml of nutrient solution (minus Fe) at 20°C in a sealed cuvette with a Clarke O_2 electrode. When the effect of SHAM was investigated, the original solution was replaced by a similar one containing 15 mM SHAM. KCN and CCCP were added directly to the vessel. After measurements, the roots were blotted dry and weighed.

Estimation of Cyt Oxidase and Alternative Path Activity. Previous results from our laboratories have shown that the contribution of the respective pathways to overall O_2 consumption in leaves and roots can be estimated using KCN to inhibit Cyt oxidase and SHAM to inhibit the alternative path (3, 6, 7). Inhibition by KCN allows the capacity (maximum possible activity) of the alternative path to be estimated, while inhibition by SHAM alone indicates the actual activity of the alternative path (*i.e.* its contribution to O_2 uptake). Since the alternative path acts as an overflow of the Cyt path—it is only engaged when the Cyt path is saturated, or inhibited by lack of ADP (8, 10)— inhibition by SHAM in the presence of uncoupler allows the capacity of the Cyt path to be estimated. On the other hand, inhibition by SHAM in the absence of uncoupler but not in its presence, shows that adenylate control of the Cyt chain occurs (2). Lack of inhibition of O_2 uptake either in the absence or in the presence of uncoupler indicates that the alternative path does not operate.

Chemical Analyses. Sugars (glucose plus fructose) were assayed enzymatically in dried and ground material (1). Nitrogen analyses were done with the Kjeldahl method, using $CuSO_4:K_2SO_4$ (1:3) as a catalyst (5). The samples were partitioned into a 3% HCl soluble fraction and an insoluble fraction.

RESULTS

Leaf Respiration and Plant Growth. Table IA shows average rates of O_2 uptake in the dark by slices cut from fully expanded leaves of the two populations. In agreement with previously reported rates of CO_2 evolution from whole attached leaves (12, 13), GL66 (the 'fast' respiring line) yielded rates approximately 40% faster than those of GL72 (the 'slow' respiring line). Neither KCN (an inhibitor of Cyt oxidase), nor SHAM (an inhibitor of the alternative path) had any significant effect on the rate of O_2 uptake by mature leaves from either line when added alone, but when these inhibitors were added together, respiration was severely inhibited (Table IA). The slower respiration rate of GL72 was reflected in the growth of the plants; the increase in dry weight of both shoots and roots with plant age was greater in GL72 than in GL66 (Fig. 1A), and fully expanded leaves were both longer and thicker in GL72 (see below). The shoot to root ratio was virtually identical in the two populations (Fig. 1B). Initial relative growth rate was also significantly higher in GL72 (219 *versus* 186 mg·g^{-1} [plant dry weight] for GL66). This agrees with the previous analyses of Robson (12, 13).

The pattern of respiration in the meristematic bases of leaves was quite different from that in the mature leaf (Table IB). The rate of O_2 uptake by slices from meristematic regions was the same in both populations, and this O_2 uptake was severely

Table I. *Mean Respiratory Rates of Leaves of Two Populations of L. perenne, Selected for Either Fast (GL66) or Slow (GL72) Mature Leaf Dark Respiration*

The concentration of KCN was 0.5 mM and of SHAM, 5 mM. Control rate represents O_2 uptake in the absence of inhibitors.

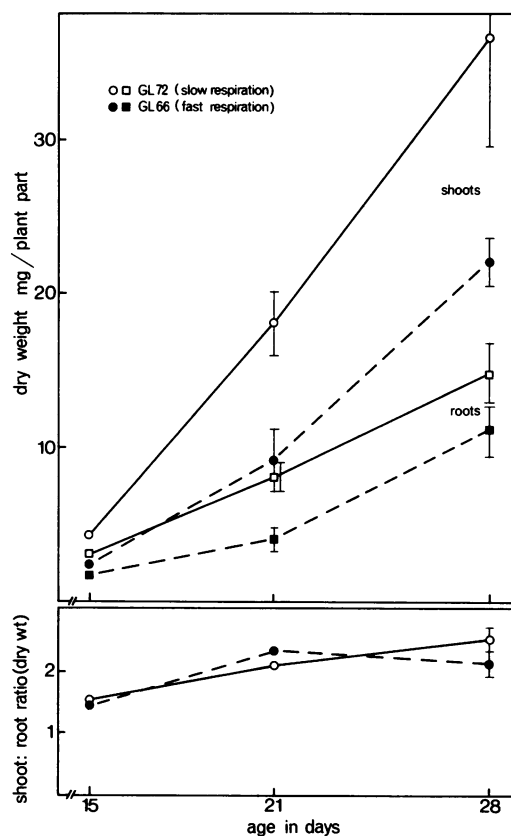| Conditions	O_2 Consumption	
	GL66	GL72
	$\mu mol \cdot (g\ fresh\ wt)^{-1} \cdot h^{-1}$	
A. Mature tissue		
Control ($n = 16$)	7.3 (0.2)[a]	5.2 (0.2)
+ KCN ($n = 6$)	8.0 (0.5)	5.4 (0.4)
+ SHAM ($n = 9$)	7.3 (0.3)	5.2 (0.3)
+ KCN and SHAM ($n = 9$)	1.0 (0.1)	0.8 (0.1)
B. Meristematic tissue ($n = 5$)		
Control	11.7 (0.5)	11.9 (0.6)
+ KCN	2.9 (0.3)	2.9 (0.4)
+ KCN and SHAM	1.6 (0.3)	1.4 (0.1)

[a] Numbers in parentheses, SE.

FIG. 1. Dry matter increments in shoots (O, ●) and roots (□, ■) of two populations of *L. perenne* L. cv S23, selected for either fast (GL66; ●, ■) or slow (GL72; O, □) mature leaf dark respiration. Means of three or five independent measures with four plants; bars represent 2 × SE.

inhibited by KCN alone. Addition of SHAM alone had no effect on O_2 uptake (Fig. 2D) but when added after KCN it caused a further inhibition (Table IB). These differences between mature and meristematic leaf tissue can be seen more clearly in Figure 2 which depicts typical individual O_2 traces. Although only results obtained with GL72 are shown, similar trends were observed with GL66. O_2 uptake by slices taken from the middle of fully expanded leaves was slower than that by slices from unexposed (yellow) leaf bases and was unaffected by SHAM and only
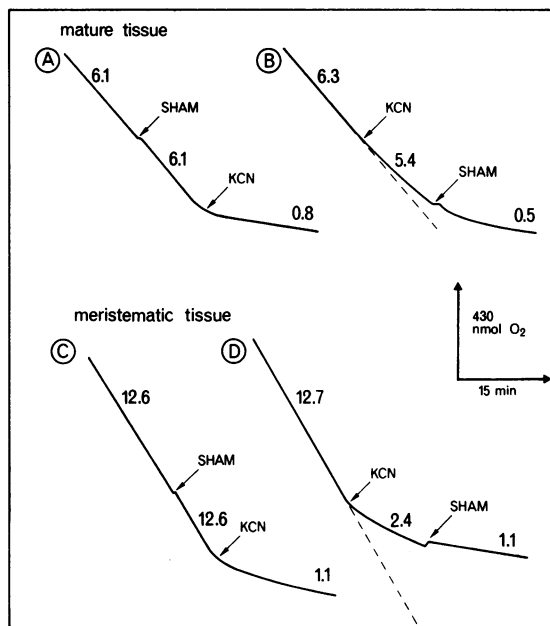
FIG. 2. O_2 uptake by mature and meristematic segments of leaves of *L. perenne* L. cv S23, population GL72. Additions as indicated were 8 mM SHAM and 0.5 mM KCN. The fresh weights of tissue in each experiment were: A, 0.18 g; B, 0.15 g; C, 0.11 g; D, 0.13 g. Numbers on traces refer to μmol O_2 (g fresh wt·h)$^{-1}$.

Table II. *Mean Soluble Carbohydrate (n = 3) and Reduced N (n = 4) Concentrations in the Leaves of Two Lolium Populations with Either Fast (GL66) or Slow (GL72) Mature Leaf Dark Respiration Rates, 4 to 6 h after the Start of the Day, after 6 to 8 h of Darkness, and after 30 h of Darkness*

Treatment	Population	
	GL66	GL72
	μmol sugar/g fresh wt	
4–6 h of photosynthesis	7.4 (0.7)[a]	5.6 (0.4)
6–8 h of darkness	6.1 (0.1)	6.2 (0.6)
30 h of darkness	8.1 (1.1)	5.9 (0.2)
	mg reduced N/g fresh wt	
Soluble	4.4 (0.5)	3.7 (0.2)
Insoluble	5.7 (0.5)	5.6 (1.0)

[a] Numbers in parentheses, SE.

slightly inhibited by KCN alone (Fig. 2, A and B). That is, although the mature tissue had substantial alternative path capacity, this path did not contribute to O_2 uptake in the absence of KCN (note that the lack of effect of KCN was not due to its poor penetration, because addition of KCN after SHAM inhibited O_2 uptake severely; Fig. 2, A and B). O_2 uptake by the leaf base slices, on the other hand, was severely inhibited by KCN alone (Fig. 2C). That is, the capacity of the alternative path in meristematic leaf tissue was much less than that in mature leaf tissue. Presumably, the alternative path capacity increases during leaf development.

Regulation of Mature Leaf Respiration. The manner in which leaf respiration in the two *Lolium* populations is regulated was investigated by the use of various effectors and substrates. It should be noted that, unlike wheat leaf respiration (3), *Lolium* leaf respiration showed no significant diurnal variation in the rate of respiration (not shown). In wheat leaves, respiration rates varied diurnally as a result of fluctuations in leaf sugar levels (1,

Table III. *Effect of Uncoupler, Inhibitors, and Substrates on Mature Leaf Respiration of two L. perenne Populations, Selected for Either Fast (GL66) or Slow (GL72) Mature Leaf Dark Respiration*

Rates are expressed as percentages of the basal rate (see Table I) and are means (n at least 4). The concentrations of effectors were: 2 μM CCCP, 40 mM sucrose, 40 mM glycine, 8 mM SHAM, and 0.2 mM KCN.

Sequential Additions	Oxygen Consumption	
	GL66	GL72
	%	
A. None	100	100
+ CCCP	122 (3)[a]	115 (1)
+ sucrose	136 (4)	131 (3)
+ SHAM	135 (3)	131 (3)
+ KCN	26 (1)	29 (1)
B. None	100	100
+ sucrose	102 (2)	103 (2)
+ CCCP	123 (2)	128 (2)
C. None	100	100
+ glycine	119 (1)	113 (1)
+ CCCP	132 (4)	132 (4)
D. None	100	100
+ sucrose and CCCP	125 (2)	124 (2)
+ KCN	108 (9)	105 (10)
+ SHAM	21 (4)	20 (5)
E. None	100	100
+ glycine	120 (3)	115 (1)
+ SHAM	120 (3)	115 (1)
+ CCCP	131 (3)	136 (2)

[a] Numbers in parentheses, SE.

3). *Lolium* leaf sugar levels, on the other hand, did not change significantly during the night or even after 30 h of continuous darkness (Table II). Consequently, further experiments were carried out only on leaves harvested near the middle of the day.

Adding an uncoupler (CCCP) stimulated the rate of O_2 uptake by both lines by approximately 20% and subsequent addition of sucrose stimulated it further (Table IIIA). Addition of sucrose in the absence of CCCP, however, had virtually no effect on respiration (Table IIIB), although adding glycine alone stimulated it by 15–20% (Table IIIC). Addition of CCCP after glycine stimulated it further (Table IIIC). These results suggest that the rate of respiration in both lines was restricted by adenylate control (*i.e.* by high cytosolic energy charge) of glycolysis, which was relieved by adding uncoupler. The stimulation observed with glycine alone, which is metabolized directly by the mitochondria, shows that electron transport *per se* was not restricted.

This regulation via glycolysis was the same in both GL66 and GL72 (Table III) showing that the faster rates of O_2 uptake which characterize GL66 (Table I) are not due simply to provision of more substrate to the mitochondria in the leaf cells.

Although sucrose plus CCCP, and glycine, stimulated O_2 uptake, subsequent addition of SHAM alone still did not inhibit respiration (Table III, D and E). That is, the alternative pathway remained disengaged even in the presence of exogenous substrates. KCN, however, inhibited respiration significantly in the presence of CCCP and sucrose (Table IIID), allowing an estimate of the capacity of the alternative path to be made. This capacity was the same percentage of total measured O_2 uptake in both GL66 and GL72, but was larger, in absolute terms, in GL66 (the faster respiring line). Since the respiration of both lines was regulated in the same manner, results of further experiments on

Table IV. *Effect of Various Substrates on Mature Leaf Respiration of GL72*

Rates are expressed as percentages of basal respiration (see Table I) and are means (*n* at least 4). Concentrations of effectors were: 30 mM sucrose, 2 μM CCCP, 30 mM glycine, 30 mM malate, 8 mM SHAM, and 0.5 mM KCN.

Sequential Additions	O₂ Consumption
A. None	100
+ malate	120 (1)[a]
+ glycine	142 (1)
+ CCCP	161 (5)
+ SHAM	159 (7)
+ KCN	13 (1)
B. None	100
+ sucrose and CCCP	122 (2)
+ malate	135 (2)
+ glycine	152 (3)
+ SHAM	150 (2)
+ KCN	14 (1)
C. None	100
+ sucrose	100 (1)
+ CCCP	120 (3)
+ glycine	146 (3)

[a] Numbers in parentheses, SE.

leaf slices are shown only for GL72 since GL66 performed similarly (Table IV).

Table IV shows the effects of adding combinations of different substrates on mature leaf slice O₂ uptake. Addition of malate stimulated O₂ uptake by 20% (Table IVA); subsequent addition of glycine stimulated it by another 20% and CCCP stimulated it by a further 20% (Table IVA). Malate also stimulated respiration when added after sucrose and CCCP (Table IVB), as did glycine (Table IVC). Addition of glycine as a third substrate after sucrose (+CCCP) and malate resulted in a further stimulation of O₂ uptake (Table IVB). The presentation of three substrates in the presence of uncoupler gave an overall increase of 50–60% of respiration, but even under these conditions SHAM alone had no effect (Table IVA and B), showing that the Cyt path was still not saturated. Nor did SHAM inhibit O₂ uptake in the presence of malate plus glycine but in the absence of uncoupler (results not shown).

The rates of O₂ uptake in leaves from both *Lolium* populations are summarized in Table V. Interestingly, the respiration rate of the meristematic leaf bases was not stimulated by any combination of substrates or uncoupler (not shown) and in GL66, O₂ uptake by mature leaf slices in the presence of a cocktail of substrates and uncoupler were at least as fast as that by the meristematic tissue (Table V).

Root Respiration. Table VI summarizes measurements of O₂ uptake made with mature roots from both populations. Very little difference in the rate of O₂ uptake in either the presence or the absence of respiratory inhibitors was observed between GL66 and GL72. Respiration in the presence of KCN was substantial and the inhibition by SHAM alone indicates that the alternative path contributed approximately 35% to the total O₂ uptake. Addition of uncoupler stimulated O₂ uptake significantly in the presence of SHAM, suggesting that the Cyt path in these roots was restricted by ADP supply, with electrons spilling over into the alternative path. Addition of KCN and SHAM together inhibited respiration by at least 90%. The degree to which the alternative oxidase contributed to overall respiration was approximately the same for both lines. The ratio of the alternative oxidase activity to the Cyt oxidase activity was 0.60 for GL66

Table V. *Summary of Leaf Respiration in L. perenne Populations Selected for Either Fast (GL66) or Slow (GL72) Mature Leaf Dark Respiration*

Rates shown were calculated from the data of Tables I, III, and IV and are means (*n* = 6–16). Residual respiration (O₂ uptake in the presence of KCN + SHAM) was subtracted from all values. Alternative path capacity was calculated from O₂ uptake in the presence of KCN, sucrose, and CCCP. The maximum Cyt path activity was taken as O₂ uptake in the presence of a mixture of substrates and CCCP and SHAM. The basal rate is the rate of O₂ uptake in the absence of exogenous effectors. For further details, see the text.

Tissue and Line	Basal Rate	Maximum Cyt Path Activity	Alternative Path Capacity
	μmol O₂ (g fresh wt·h)⁻¹		
A. Mature tissue			
GL66	6.3 (0.2)[a]	11.7 (0.4)	6.4 (0.2)
GL72	4.4 (0.4)	8.3 (0.3)	4.5 (0.5)
B. Meristematic tissue			
GL66	10.1 (0.5)	10.1 (0.5)	1.3 (0.3)
GL72	10.5 (0.6)	10.5 (0.5)	1.5 (0.1)

[a] Numbers in parentheses, SE.

Table VI. *Root Respiration of Different L. perenne Populations Selected for Either Fast (GL66) or Slow (GL72) Mature Leaf Dark Respiration*

The concentration of SHAM was 15 mM, that of KCN, 0.5 mM, and of CCCP, 2 μM. Rates shown are means (*n* = 4). The plants used were 3 months old.

Conditions	Oxygen Consumption			
	GL66	GL72	GL66	GL72
	μmol (g fresh wt·h)⁻¹		%	
Basal rate	4.0 (0.4)[a]	3.8 (0.3)	100	100
+ KCN	2.3 (0.1)	2.8 (0.2)	58	74
+ SHAM	2.5 (0.1)	2.5 (0.2)	63	66
+ SHAM and CCCP	3.6 (0.2)	3.5 (0.2)	90	93
+ SHAM and KCN	0.3 (0.1)	0.4 (0.1)	9	10

[a] Numbers in parentheses, SE.

and 0.52 for GL72.

Chemical Composition of Leaf Material. There were no significant differences in the concentration of soluble sugars (glucose plus fructose) between the two populations (Table II). At the end of the night, and also after 30 h of darkness, the soluble sugar concentration was the same as that after several h of photosynthesis.

Both the soluble ('amino acids') and the insoluble ('protein') reduced *N*-concentrations were the same in both populations (Table II).

Anatomical Studies. The leaves showed the typical grass leaf anatomy, with ridges and grooves at the adaxial side. The vascular bundles in the ridge were surrounded by two bundle sheaths. The transverse distance between the bundles was constant and the same for leaves of both populations. Consequently, the wider leaves had more ridges and bundles than the narrower ones. In the grooves, the epidermal cells were enlarged, forming conspicuous bulliform cells. The mesophyll showed no differentiation into palisade and spongy parenchyma. It consisted of transverse plates, separated by more or less irregular intracellular spaces. Branched cells were numerous in the mesophyll.

The leaves of GL72 were thicker than those of GL66. The average thickness of GL72 was 0.28 mm (ridges) and 0.13 mm (grooves), *versus* 0.17 and 0.10 in GL66. Also, the vascular

bundles in GL72 were somewhat wider. The only distinct anatomical difference was the volume of intercellular space in the mesophyll. In longitudinal leaf sections, the amount of intercellular spaces was 47 (ridges) and 60% in GL72 *versus* 22 and 50% in GL66. Thus, in the thicker leaves of GL72 only the intercellular spaces were larger. The mass of the cellular material was approximately the same in both populations. Also, the specific leaf weight (g fresh weight per m^2) was the same for leaves of the contrasting populations.

DISCUSSION

Fast Respiring *versus* Slow Respiring Populations. The results presented here confirm that the *L. perenne* GL66 population has higher rates of mature leaf respiration and slower growth compared to GL72. However, the respiration rate of meristematic leaf tissue, and the roots, is approximately the same in both. Thus, the increased biomass produced by GL72 reflects principally more efficient use of assimilated carbon in the fully expanded leaves. However, the similarity in the rate of root respiration also warrants further investigation: the faster growing roots are expected to respire faster.

The difference in dark respiration in mature leaves between the two lines is due to a difference in total respiratory capacity rather than a difference in the manner in which respiration is regulated. The lack of effect of added sucrose (alone) on O_2 uptake and the stimulation by uncoupler, glycine, and malate in both lines suggests that in the mature leaf, respiration in the dark is limited by substrate supply to the mitochondria. This limitation is brought about by adenylate control of glycolysis (the stimulation of O_2 uptake by glycine and malate in the absence of uncoupler, and the lack of effect of SHAM, an inhibitor of the alternative path, show that Cyt path activity *per se* was not limited).

Since SHAM had no effect on uncoupled respiration even when a cocktail of substrates was added (Table IV), it was not possible to estimate the full capacity of the Cyt chain (see "Materials and Methods"), but the maximum activity measured was greater in GL66 than in GL72 (Table V). This is also true for the alternative path, which did not contribute to O_2 uptake under the conditions imposed in our experiments.

Comparison of the O_2 uptake rate measured in the absence of effectors to the combined estimated capacities of the alternative and Cyt pathways (Table V) shows that in both lines only approximately one-third of the total respiratory capacity of the mitochondria was used in the dark. However, since the total capacity of GL66 is higher than that of GL72, O_2 uptake in the dark was faster in GL66. Whether the greater electron transport capacity of GL66 is due to higher specific mitochondrial activity or to more mitochondria per g fresh weight cannot be assessed yet. The similar effects of substrates and uncoupler on the respiration of the two lines shows that glycolysis limits O_2 uptake in both. Control of glycolysis presumably involves high cytosolic ATP:ADP ratios. Such high ratios have indeed been observed, using ^{31}P nuclear magnetic resonance spectroscopy (11).

It therefore appears that the higher O_2 consumption by GL66 reflects faster turnover of cellular ATP. This in turn implies that energy consuming processes in mature leaf cells in the fast respiring population are less efficient than those in the slow one. Processes such as metabolite translocation and protein turnover are possibly involved here. The analysis of respiratory control outlined above also suggests that there are unlikely to be severe penalities incurred by selection for slow respiration in *Lolium*.

Possible Causes of a Higher ATP Demand in the High Respiration Population. The present results do not provide a definite clue to explain the increased ATP demand of GL66 plants. However, the similarity in chemical composition (Table II) and in anatomy indicate that the increased demand for ATP is due to neither an increased net rate of protein synthesis nor increased distances for the transport of sucrose from the chloroplast to the phloem.

Regulation of Respiration in Different Tissues. The stimulation of O_2 uptakes in mature leaf slices by malate, glycine, and uncoupler indicates that substrate presentation to the mitochondria limited overall respiration in this tissue and further indicates that different pathways are used for the metabolism of different substrates. Sucrose is metabolized via glycolysis, giving rise to pyruvate, while glycine and malate presumably enter the mitochondria directly to be oxidized, respectively, by glycine decarboxylase and NAD-malic enzyme, thus bypassing adenylate control of glycolysis. However, it should be noted that it is also possible that different substrates have their effects on different cell types, since the leaf is a heterogeneous tissue, although the bulk of O_2 uptake probably occurs in the more numerous photosynthetic cells.

The lack of saturation of the electron transport chains in the presence of several different substrates probably reflects restriction on substrate entry into the leaf slices or sequestration of some substrates by compartments other than the mitochondria. It is interesting to note that although endogenous sugar levels were quite high in these leaves, exogenous sucrose stimulated O_2 uptake when uncoupler was present. This suggests that not all of the endogenous sugars were available to respiration, implying control by compartmentation.

The control of respiration in *Lolium* leaves is very different from that found in wheat leaves. In the latter tissue, respiration rate is controlled in the first instance by the concentration of sugars in the leaf (3). When sugars are plentiful, however, control of the Cyt chain activity by ADP:ATP becomes evident in wheat leaves, with electrons from substrate oxidation spilling over into the alternative path (2). That is, in wheat leaves, substrate provision to the mitochondria exceeds the activity of the Cyt chain which is restricted by adenylates. The reverse situation occurs in *Lolium* leaves. It is interesting to note that malate stimulates *Lolium* leaf respiration (Table III) but apparently not that of wheat leaves (J. Azcon-Bieto, personal communication). It is tempting to speculate that in wheat leaves malate is produced endogenously via phosphoenolpyruvate acid-carboxylase and malate dehydrogenase in the cytosol during metabolism of sugars, bypassing adenylate control points of glycolysis and allowing expression of the alternative path (6). Clearly, a more detailed comparison of these tissues is warranted.

The pattern of respiration also varies from tissue to tissue within a given plant species. Respiration rates of the meristematic leaf base of *Lolium* were higher than those of the mature leaf (Table I) and did not respond to uncoupler or exogenous substrates, suggesting that respiratory flux was near maximal in this tissue. This presumably reflects greater demand for ATP in meristematic regions, an idea supported by the very low capacity of the alternative path in the leaf base (Table V). It should be noted that measurements were made on segments taken from within the leaf sheath and hence the tissue was probably not photosynthetic (it was a very pale yellow color). Thus, lack of effect of glycine here may have been due to the absence of glycine decarboxylase. Development of the alternative path in the upper portions of the leaf may have been a consequence of greening rather than maturation, since alternative path capacity in (green) bean and pea leaves did not change during leaf development (4). Root respiration provided a more dramatic illustration of the flexibility of the respiratory control in plant tissues (Table VI). Here, SHAM alone inhibited respiration substantially, showing that the alternative path contributed to O_2 uptake, and subsequent addition of uncoupler relieved this inhibition. This indicates that root respiration was, at least in part, restricted by adenylate control of the Cyt path, with glycolytic flux not

matched to the cell's energy demand. This is in marked contrast to the leaf where ATP turnover strictly limits glycolysis (see above). Thus, care must be exercised when making generalizations about the nature of respiratory control in plants.

Finally, it is interesting to note that the respiratory capacity of mature leaves was not fully realized in the dark in our experiments. Similar observations have been made with other tissues (2–4). This may indicate that mitochondrial respiration is faster in the light than in the dark when supply of substrates may be greater (1). This in turn implies that the nature of respiratory control is different in the light from that in the dark.

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